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STUDY ON THE PINEAL ORGAN OF COMMON CARP (*CYPRINUS CARPIO*) AND SILVER CARP (*HYPOPHTHALMICHTHYS MOLITRIX*) IN TAIWAN

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ABSTRACT

The pineal organ is well-developed in common carp (*Cyprinus carpio*) at the adult stage, but in silver carp (*Hypophthalmichthys molitrix*) it is still underdeveloped even at age four. The pineal organ of these two locally produced carps is developed as an evagination from the anterior wall of the velum transversum and located between the posterior poles of cerebral hemispheres and the optic lobes. It grows with age, and the final appearance of this organ in mature common carp shows complicated and tree-like glandular structure with numerous anastomosing pineal tubules. These tubules are formed by a single layer of columnar ependymal cells which have conspicuous large and oval nucleus, of which both the nucleolus and irregular clumps of chromatic materials can be easily observed. Cytoplasm of these cells is of homogeneous in appearance, filled with a great amount of thread-like substances. Two types of scattered pigmented-cells can be found within the space of pineal tubule, and they are connected by the threads of the sticky coagulable fluid in the tubule. Venous sinusoids, formed entirely by a thin layer of endothelium, are occasionally found in the tubule. No arterioles can be seen, and this strongly indicates that the supply of blood to this organ is completely from venous system.

The silver carp is one of the most important cultured fishes in Taiwan. Because this locally produced carp does not spawn in the cultured fish pond, many fish culturists have tried to induce spawning with hormone injection but so far without any satisfactory result. It has been found that the pattern of sexual maturation of the silver carp is complicate[6]. As the hypophyseal function of this fish has been studied to be normal[7], and many previous investigators have already indicated that the development of this organ coincides with the development of gonad[5], it is, therefore, worthwhile to know the condition of the pineal organ of this fish. The common carp can naturally spawn in fish pond, so that its pineal organ is also studied for comparison. The present investigation deals with the differences between the pineal organs of these two species of carps with respects to the original development, morphological appearance as well as histological structure.

MATERIALS AND METHODS

All the silver carps used in this

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investigation were caught in the fish culture pond of National Taiwan University and the common carps were bought from the fish market. These two species were subdivided into three different categories: the immature group of one year old, mature group of 2 and 3 years old and the fully mature group of the age over 4, respectively. Two males and two females in each category were dissected bimonthly. The pineal organ, especially of the immature group, is too small to be seen clearly, therefore the whole brain must be removed. All the brains were immediately fixed in Zenker's solution after its removal. Sagittal and serial section of each pineal organ were made by paraffin method at 4 microns in thickness, and counter stained in Mallory's triple stain and haematoxylin-eosin. Three methods were adopted to study the maturity of the fishes namely: 1) by gross observation on the condition of gonad, 2) by comparing the index of maturation (gonad weight/body weight and gonad weight/standard length), and 3) by studying the size composition of the ovarian eggs.

OBSERVATIONS

General Morphology

The pineal organs of these two species of carps lie in the median line on the dorsal surface of brain between cerebral hemispheres and optic lobes. The general feature of this organ in common carp shows almost spherical in appearance (Fig. 1.), larger in the mature group with an average diameter of 3-4 mm and smaller in both fully mature and immature groups with an average diameter of 2-3 and 1-3 mm respectively. The surface of the whole organ is covered by a thin layer of connective tissue which continues with the meninges of the brain. As for the silver carp, it is very underdeveloped even at age four (Fig. 2.) which can be observed only under a microscope. Its appearance composes of a single layer of columnar cells and an inner undifferentiated mass. The whole structure has the measurement of 3 mm in length and less than 0.5 mm in width as shown in Fig. 2.

Development

The development of this organ in both of these two species is originated from the evagination of the anterior wall of velum transversum. This origin is quite similar to that of reptilian and amphibian[3,4,&9]. From all the examined specimens, it can be clearly observed that on the surface of the velum transversum, there are two layers of cells, the outer one of which is composed completely of a single layer of columnar cells which will later become the columnar ependymal cells of the pineal organ to form the pineal tubules when they migrate anteriorly (Fig. 3 & 4.). The inner layer is composed of many oval-shaped cells which will later beccme the scattered pigmented-cells as well as the venous sinusoids within the space of the pineal tubules. The columnar cells of the outer layer of velum transversum separete gradually from the velum transversum when they migrate forward at the early stage of the formation of this organ to form a structure so simple that it is composed of a single layer of these cells. Then this simple structure grows by the way of folding and refolding to form the tree-like pineal tubules. This results in the increase of its size and eventually becomes a highly complicated and vascular structure with numerous fine pineal tubules. The columnar cells of the pineal tubules are velum transversum in origin but a little larger in size than those originally located ones.

The serial sections of this organ show that the scattered pigmented-cells within

the tubules are the immigrants of the inner layer of velum transversum. At the beginning of the formation of this organ, when the columnar cells migrate anteriorly to form the tubules, the oval-shaped cells of the inner layer also moved anteriorly following the columnar cells as shown in Fig. 3 & 4. When this organ grows to maturity, these oval-shaped cells are situated everywhere in the space of the tubules.

The development of the pineal organ in these two species is quite different. In silver carp, it appears at the young stage forming a very simple stucture composed of only one single layer of columnar cells and no further development takes place even over age 4. Gonads of all these sectioned silver carps had been used for the study of sexual maturation and the result showed that they were all immature. The pineal organ of common carp, on the other hand, was found highly differentiated at the adult stage. It never showed any sign of degeneration. In the sections of the pineal organ of a 3 years old fish, it had already developed into a fully mature and highly complicated vascular organ. All of these sectioned carps were found to reach the sexual maturity.

HISTOLOGY

The histological study of the pineal organ has dealt only with the common carp because only this species of carp used in the present investigation has a mature and well-developed organ. Four types of cells were found in the structure of a fully mature pineal organ. They were: (1) columnar cells, (2) scattered pigmented cells, (3) endothelial cells of the venous sinusoids and (4) red blood cells.

The venous sinusoids are made up entirely of a thin layer of endothelium. The endothelial cells are flattened and form the venous sinusoids with various size depending upon the number of these endothelial cells. Among them some permitted only a single red blood cell to pass through (Fig. 5.) but some possessed a comparatively larger lumen. No connective tissue and smooth muscle cell can be found outside the venous sinusoids. These venous sinusoids were located almost in each pineal tubule and they communicated with each other at the open end of the pineal tubules.

The columnar cells are the most important cells which render this organ to be a branched and rebranched structure. All the clear open spaces of the pineal tubules are completely bound by them. These columnar cells, termed by many previous investigators as ependymal cells[2,4, & 10], are nearly 20 microns in length and about twice of its width. It can be obviously seen that a large oval nucleus, about 6-8 microns in diameter, is situated at the center of the cell. The nuclear membrane, with deep brown stain, is rather prominent. The thread-like chromatic materials are irregularly clumped throughout the whole nucleoplasm but are present a little more densely along the margin of the nuclear membrane. The round-shaped nucleolus is usually present, sometimes at the center but occasionally at the margin of the nucleus. Mitochondria which are filament-like threads and visible under a dimly lighted condition, were usually concentrated near the inner margin of cell membrane. Cytoplasm of these ependymal cells appeared homogeneous and no other particulate cellular structure found under a light microscope. No intercellular space between any two adjacent ependymal cells was present but the space between the outer surface of two neighbouring tubules had a narrow canal. This canal was filled with connective tissue and empty vacuoles (Fig. 5.). These highly developed and complicated

vascular tubules of the pineal structure strongly indicate that the pineal organ is a glandular structure and its secretions from the ependymal cells are absorbed by the venous sinusoids and translocated.

The internal space of the pineal tubule was filled with the albuminous and transparent coagulable fluid. Two types of pigmented cells were found scattered around there (Fig. 8.). These pigmented-cells were connected by the threads of the above mentioned sticky fluid (Fig. 7.). The first type of these cells was comparatively large and spherical-shaped, about 8 microns in diameter, a little larger than the nucleus of the ependymal cell. The cytoplasm stained with orange G appeared rather homogeneous, only small pigmented-dots were found there. The nucleus, oval-shaped and pale brown in color, located mostly at the margin of the cytoplasm, was seldom found in the center of the cell. Chromatic material and nucleolus were not clearly visible. The other type of the scattered pigmented cells was very small in size, nearly the same size of the nucleus of former type. Cell membrane was not distinguishable from cytoplasm. The nucleus deeply stained appeared dark brown and usually pigmented. Probably it is the immature cell of the former type because occasionally another transitional type of pigmented-cell having a median size and color staining character between the above mentioned two was found.

DISCUSSION

Many investigators described that a large vesicle was present in the center of the pineal organ in *Lacertilia*[10] and that two vesicles, a dorsal large optic vesicle and a ventral small astrium, in *Geotria australis*[2]. However, no vesicle has been found in fishes of the present investigation. No retina can be seen, and the whole pineal organ has never shown any resemblance to an"eye" which had been reported in cyclostomes and amphibians[1 & 10].

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REFERENCES CITED

1. EEARD, J. 1889: Morphological studies of the parietal eye of the cyclostome fishes. Journal of Microscopical Science 29: 55-73.
2. DENDY, ARTHUR 1907: On the parietal sense-organ and associated structure in the New Zealand lamprey (*Geotria australis*). Journal of Microscopical Science 51: 1-29.
3. _____ 1910: On the structure, development and morphological interpretation of the pineal organ and adjacent parts of the brain in the Tuatata (*Sphenodon punctatus*). Anatomischer Anzeiger 37: 453-462.
4. EYCLESHYMER, ALBERT C. 1897: Paraphysis and epiphysis in Amplystoma. Anatomischer Anzeiger 7: 215-217.
5. KITAY, JULIAN I. and MARK D. ALTSCHULE 1954: The pineal organ. Harvard University Press, Cambridge, Mass., U.S.A.
6. KUO, CHING-MING, I-HSIU TUNG, TIEN-HSI TAN and FAH-HSUEN LIU 1965: The physiology of reproduction of some teleost fishes: Part I. Study on the sexual maturity of silver carp (*Hypophthalmichthys molitrix*). Rpt. of the Inst. of Fish. Biol. of MOEA and NTU. Vol. II, No. 1, pp. 46-52.
7. LIU, FAH-HSUEN and TIEN-HSI TAN 1963: The physiology of roproduction of some teleost fishes: Part II, Study on the hypophysis of silver carp (*Hypophthalmichthys molitrix*). Ball. Inst. Zool., Academia Sinica 2: 37-43.
8. LEWIS, F. T. 1904: The question of sinusoids. Anatomischer anzeiger 15:261-279.
9. MINOT, C. S. 1901: On the morphology of the

pineal organ based upon its development in Acanthias. American Journal Anatomy 1: 81-98.
10. SPENCER, W. BALDWIN 1887: On the presence and structure of the pineal eye in *Lacertilia*. Journal of Microscopical Science 27: 165-238.
11. WARREN, JOHN 1905: The development of the paraphysis and the pineal region in *Necturus maculatus*. American Journal of Anatomy 5: 1-27.

Fig. 1. A well-developed pineal organ of a mature Common carp, 4 years old. ×100.

Fig. 2. A pineal organ of a Silver carp, 4 years old. ×100.

Fig. 3. Showing two layers of cells, the outer columnar ependymal cells and the inner oval-shaped cells, attached at the mid-dorsal surface of the velum transversum. ×1940.

Fig. 4. Showing the columnar ependymal cells separated gradually from the velum transversum. ×1940.

Fig. 5. Showing the smaller venous sinusoids in the space of pineal tubule and the connective tissue with empty vacuoles between the walls of pineal tubules. ×1940.

Fig. 6. Showing the comparatively large venous sinusoid in the space of pineal tubule. ×1940.

Fig. 7. Showing the threads of the coagulable fluid in the space of pineal tubule. ×1940.

Fig. 8. Showing two types of scattered pigmented cells in the space of pineal tubule. ×1940.

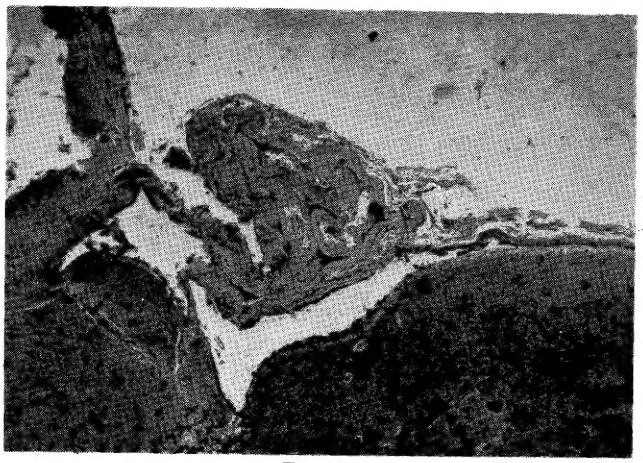

Fig. 1.

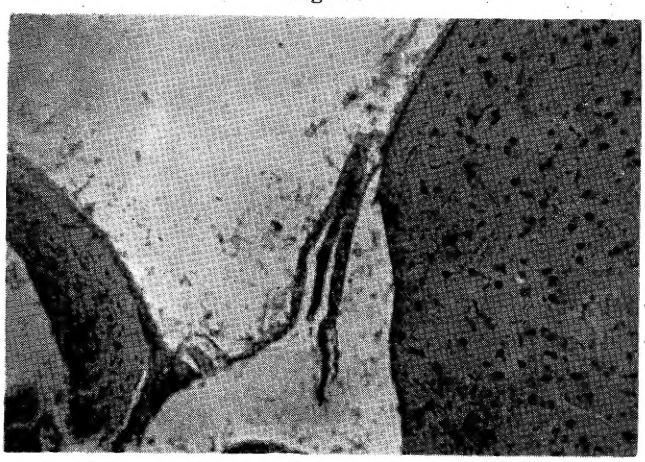

Fig. 2.

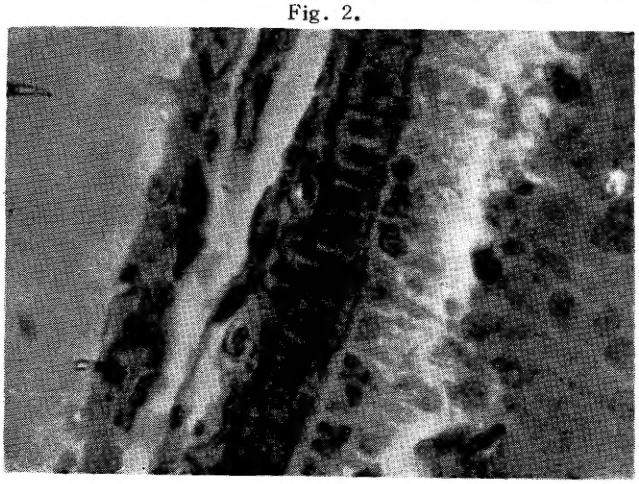

Fig. 3.

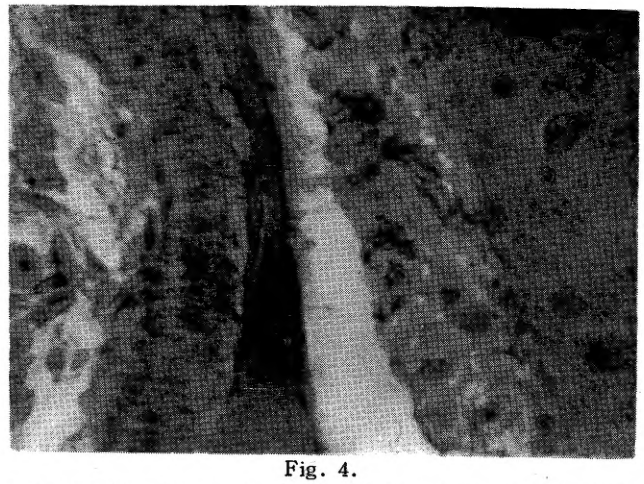

Fig. 4.

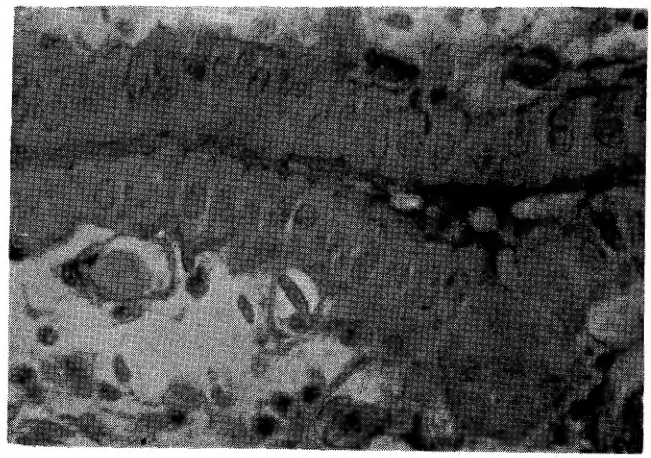

Fig. 5.

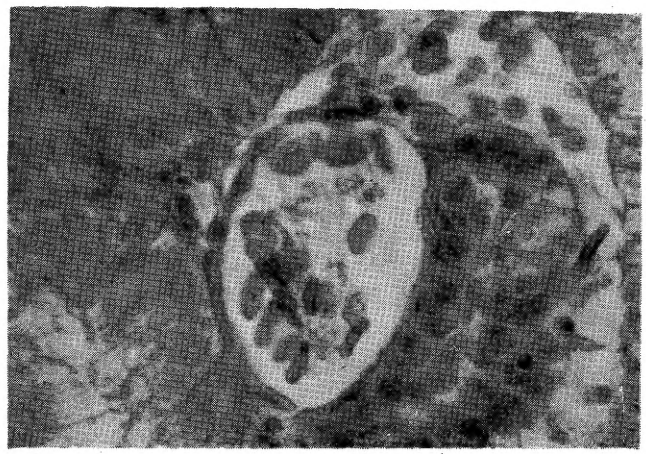

Fig. 6.

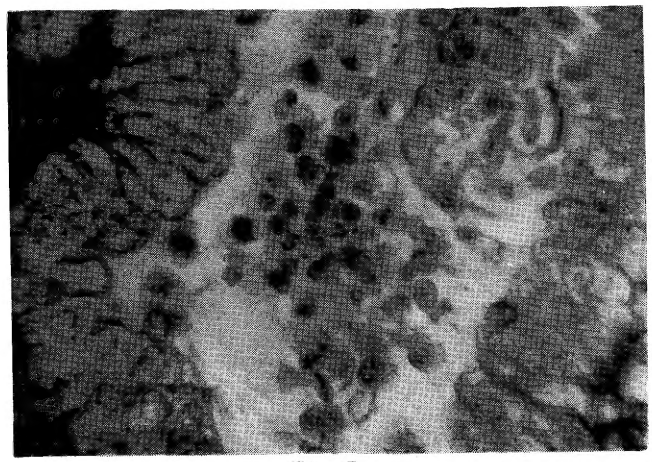

Fig. 7.

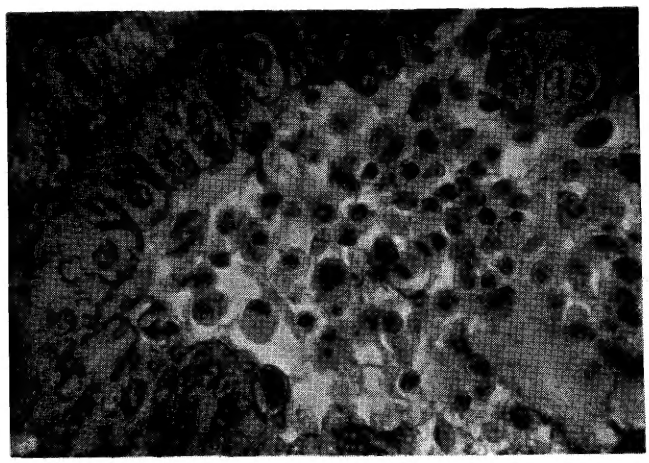

Fig. 8.